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BACTERIAL DECONTAMINATION OF FIELD DENTAL UNITS.(U)
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A portable Army Field Dental Unit was tested for bacterial contamination levels and methods to minimize contamination were explored. Decontamination by flushing or drying did not effect great changes in bacterial contamination but the addition of 3% H_2O_2 in a 1:100 ratio to water inoculated with *Pseudomonas aeruginosa* resulted in the elimination of cultivable organisms.

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(6) BACTERIAL DECONTAMINATION OF FIELD DENTAL UNITS.

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INTRODUCTION

The waterlines of dental units and accessory equipment are often heavily colonized by bacteria.[2,4,5,7,8] Although microorganisms found in the water are usually considered to be nonpathogens, their potential to infect debilitated patients has been recognized.[2] Recent studies at the U. S. Army Institute of Dental Research have shown a high level of contamination of dental units attached to communal water supplies and have shown the value of flushing lines for two minutes prior to use each day in order to greatly decrease bacterial contaminant concentrations.[5] Practical methods of eliminating the microflora from dental treatment units have not yet been developed.

Field dental units used by the U. S. Army utilize closed water systems which deliver water from a refillable reservoir. Decreased contamination of waterlines would occur if this reservoir was always sterile, and always filled with sterile water. Because of the logistical burden involved in the maintenance of a continual supply of sterile water, this study was undertaken to explore alternative solutions to the problems of water system contamination. This report summarizes current efforts to find those measures which would decrease the rate or level of bacterial contamination of the water in portable field dental units.

MATERIALS AND METHODS

The water system of a portable dental operating and treatment unit* (Fig. 1) was tested in a series of experiments.

* Encore, Inc., Portland, Oregon

1. A communal water supply was used to fill the reservoir of the dental unit which had been previously used in the field. Water samples were taken from the reservoir, the unit water syringe, and the high speed handpiece at nine day intervals for 36 days.

2. In an attempt to sterilize the water system the unit was filled with 2 per cent alkaline glutaraldehyde** for 16 hours. To remove the residual sterilant, the unit with accessories was flushed twice with 500 ml sterile tap water. Sterilized tap water was also used to fill the unit reservoir and bacteriological samples were taken at weekly intervals for five weeks.

3. In an experiment designed to test the rate of increase in bacterial colonization of the water system, the unit was again sterilized with 2 per cent glutaraldehyde. The system was then filled with water containing an 18 hour culture of *Pseudomonas aeruginosa* (ATCC #27853†). The resulting concentration of *P. aeruginosa* approximated 6.0×10^2/ml. Water samples were obtained a 0, 7, 10, 13, 16, and 20 days.

4. The efficacy of flushing as a sole means of decontamination of dental unit reservoir and waterlines was also tested. The waterlines of the unit, heavily contaminated with *P. aeruginosa* ($>1 \times 10^4$/ml), were emptied and flushed twice with 500 ml tap water and samples were obtained at 0, 3, 5, and 12 days.

5. Because drying is known to kill many microorganisms, the contaminated unit and waterlines were allowed to remain empty for 72 hours. The unit was then filled with sterile water in an attempt

** Cidex - Arbrook, Inc., Arlington, Texas
† American Type Culture Collection, Rockville, Maryland

to quantitate residual microorganisms, and samples were taken at days 0, 3, 7, and 11.

6. In an effort to find the most practical method of waterline decontamination, the use of 3 per cent hydrogen peroxide was initiated. Water contaminated with *P. aeruginosa* at concentrations of 2.75×10^3/ml to 7.50×10^4/ml was placed into the dental unit reservoir. Three per cent H_2O_2 was added to the reservoir in a 1:100 ratio and samples were obtained from the unit at 0.5 hours, 6 hours, and then daily for 7 days. Six trials were performed.

7. Finally, a comparison of the effects of various concentrations of H_2O_2 on the viability of *P. aeruginosa* was made. Appropriate amounts of 3 per cent H_2O_2 were added to test tubes containing aqueous suspension of *P. aeruginosa* (1×10^5/ml) in order to obtain 1:200, 1:100, 1:50, and 1:25 dilutions of 3 per cent hydrogen peroxide. Suspensions containing the same concentrations of organisms but no hydrogen peroxide served as controls. The viability of the test microorganisms was determined at 1, 5, 10, and 30 minutes in two trials.

Throughout all experiments, during each water sampling, approximately 2 ml of water was aseptically collected. All samples were serially diluted using sterile tap water and 0.1 ml of aliquots of the solutions were plated in duplicate on trypticase soy agar‡ plates and cultured aerobically for up to 5 days at 37°C. Colony forming units (CFU) were then counted and averaged.

‡Difco Laboratories, Inc., Detroit, Michigan

RESULTS

Immediately after tap water was placed into a previously used dental unit, water samples from the reservoir yielded 3.85×10^2 bacteria/ml. No bacterial growth could be detected in water samples from the high speed handpiece or water syringe. When assayed at 9, 18, 27, and 36 days the level of contamination in the reservoir increased two to three fold. Water from the handpiece and syringe, when cultured at the same time periods, revealed bacterial counts well exceeding those in the reservoir (Fig. 1).

After the unit had been sterilized with 2 per cent alkaline glutaraldehyde, and subsequently flushed and filled with sterile tap water, culturing of water samples over a five week period consistently revealed less than 10 CFU/ml (mean CFU = 1.42 ± 2.09).

Inoculation of *P. aeruginosa* into a sterile water system resulted in an increase of bacterial counts in the reservoir from 6.0×10^2/ml at day 0 to 1.7×10^5/ml at day 13 (Fig. 2). Although aliquots taken from the handpiece and syringe immediately after contamination showed no bacteria, succeeding samples revealed increasing counts which exceeded 2.0×10^6/ml on the 16th day of the trial.

Flushing the contaminated dental unit (>6×10^4 *P. aeruginosa*/ml) with tap water resulted in the decontamination of the reservoir (no cultivable CFU) and decreased counts in the syringe and handpiece on day 0 (Fig. 3). Subsequent samples, however, showed increased levels of contamination in the reservoir, syringe, and handpiece.

After sterile water was placed into the reservoir of the unit which had remained empty for the preceding 72 hours, no bacteria could be recovered from the water in the reservoir. However, water samples collected from the handpiece and syringe showed high bacterial counts.

Dental unit contamination with *P. aeruginosa* was eliminated by the addition of 3 per cent H_2O_2 to the reservoir. No bacteria were recovered from the reservoir, or from the syringe or high speed handpiece waterlines.

The addition of varying amounts of H_2O_2 to equal concentrations of *P. aeruginosa* demonstrated differences in the rate of decrease of bacterial counts. All microbial suspensions to which H_2O_2 was added revealed no growth at 30 minutes, while the greatest concentration (1:25) showed the earliest reduction of bacterial numbers (Table I). In both trials the number of bacteria in the control suspension remained constant or increased.

DISCUSSION

In civilian or military dental practice, whether in a clinical or nonclinical environment, there is an obvious need to use water which is free from physical or chemical contaminants. The removal of microbial contaminants is of the utmost concern, particularly in the treatment of wounded or debilitated patients.[5] Portable field dental units are used not only in routine dental care but also in emergency treatment, including high priority oral surgical procedures. The removal of the microflora from water supplies is mandatory under these conditions.

Some attempts to decontaminate the water in dental units have included filtration,[4] iodination (unpublished data), chlorination,[1] and various other physical and chemical modalities.[6] No practical system for the decontamination and maintenance of a microbe-free water supply is yet available.

This study demonstrates some fundamental conditions affecting the level of bacterial contamination of field dental treatment units:

1. The use of tap water from "safe" water supplies results in great increases in bacterial contamination of dental units (Fig. 2).

2. Dental unit contamination increases to a relatively stable, high level in water reservoirs, and even higher levels in the waterlines of the syringe and high speed handpiece (Fig. 2 and 3). These high levels may be a result of the ability of bacteria to colonize the inner surfaces of the small diameter plastic tubing of the waterlines.

3. Flushing the dental unit causes an immediate, short term drop in bacterial concentrations but does not result in a lasting decrease of bacterial numbers (Fig. 4).

4. On the basis of finding microorganisms in water samples from units which remained empty for 72 hours prior to sampling, it appears that drying, which is known to effect the death of gram negative microorganisms, may not have occurred within the small bore waterlines (Fig. 5).

5. Hydrogen peroxide, although not equally bactericidal for all microorganisms,[3] has been shown to be a practical method for the elimination of some microorganisms which colonize dental units. Hydrogen peroxide is not known to be harmful to patients and it is concluded that a 1:100 dilution can be a practical, safe, efficient method of eliminating microorganisms from water supplies.

CONCLUSION

High levels of bacterial contamination exist in the water systems of portable field dental units. The results of our studies indicate that the addition of 3% hydrogen peroxide to water reservoirs in a 1:100 ratio effectively prevents the bacterial contamination of portable field dental unit water systems, and therefore this procedure is recommended to assure delivery of bacteria-free water to patients during dental treatment.

* * * * *

MILITARY DISCLAIMER

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U. S. Army Medical Department.

Table 1. Effect of H_2O_2 on Growth of *Pseudomonas aeruginosa*.*

3% H_2O_2 Dilution	BACTERIAL COUNT (CFU/ml)							
	200		100		50		25	
TRIAL:	1	2	1	2	1	2	1	2
Time (Min)								
1	9.05×10^3	6.9×10^4	4.80×10^4	7.10×10^4	6.00×10^3	5.10×10^4	9.05×10^2	2.28×10^4
5	1.97×10^3	TNTC†	7.75×10^3	7.30×10^4	9.30×10^3	1.28×10^4	0	2.04×10^3
10	6.25×10^2	TNTC	1.18×10^3	3.50×10^4	0	1.08×10^4	0	2.80×10^2
30	0	0	0	0	0	0	0	0

*Untreated suspensions of *P. aeruginosa* contained approximately 1×10^5 CFU/ml initially, and 1×10^6 CFU/ml after 24 hours.

†Too numerous to count at the dilution performed.

LEGENDS

Figure 1. ENCORE portable field dental unit. R denotes Reservoir.

Figure 2. Bacterial counts after filling portable dental unit with tap water.

Figure 3. Bacterial counts from a portable dental unit after filling with water containing 6.0×10^2 *Pseudomonas aeruginosa* 1 ml.

Figure 4. Bacterial counts after *Pseudomonas aeruginosa* contamination and tap water flushing.

Figure 5. Bacterial counts from a portable dental unit, experimentally contaminated and emptied after 72 hours.

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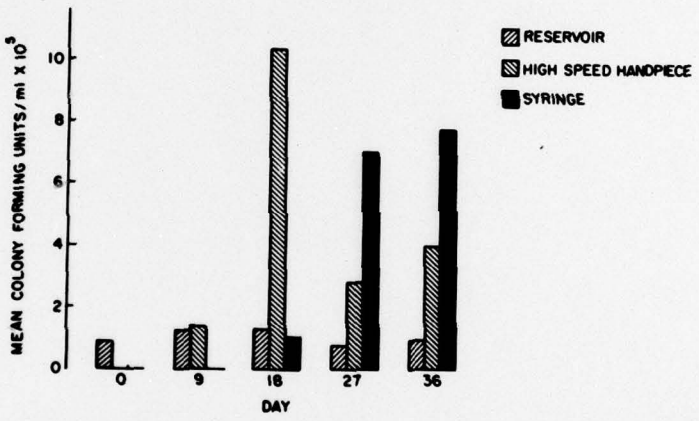
MEAN COLONY FORMING UNITS/ml X 10³
0
2
4
6
8
10
0
9
18
27
36
DAY
RESERVOIR
HIGH SPEED HANDPIECE
SYRINGE

FIG. 2

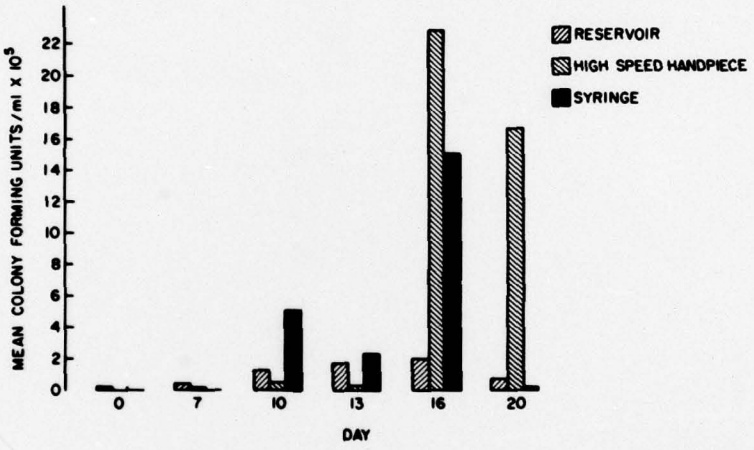
MEAN COLONY FORMING UNITS / ml X 10^5
0
2
4
6
8
10
12
14
16
18
20
22
0
7
10
13
16
20
DAY
RESERVOIR
HIGH SPEED HANDPIECE
SYRINGE

FIG. 3

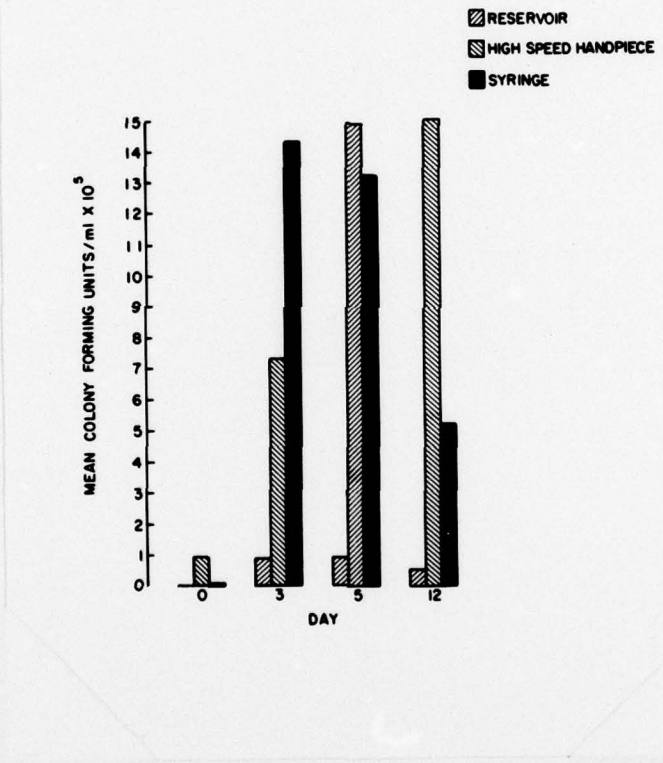
RESERVOIR
HIGH SPEED HANDPIECE
SYRINGE
MEAN COLONY FORMING UNITS/ml X 10^5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0
3
5
12
DAY

FIG. 4

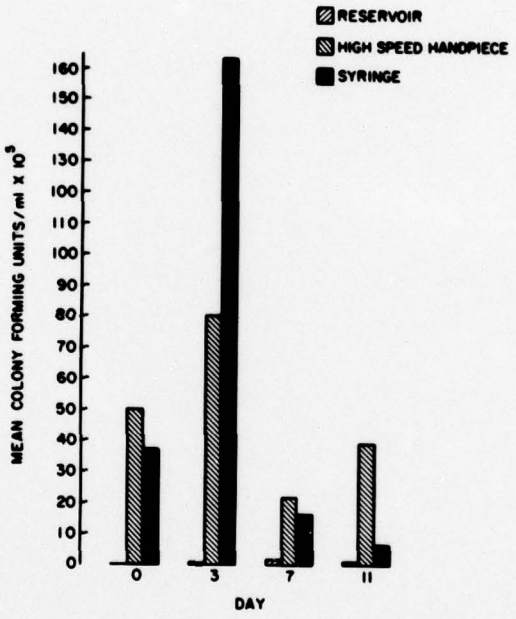
RESERVOIR
HIGH SPEED HANDPIECE
SYRINGE
MEAN COLONY FORMING UNITS/ml X 10³
160
150
140
130
100
110
100
90
80
70
60
50
40
30
20
10
0
0
3
7
11
DAY

FIG. 5